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STANDARD PATENT

I, Bruce Ian Murray, Commissioner of Patents, grant a Standard Patent with the following particulars:

Name and Address of Patentee:

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(57) Described herein are high affinity nucleic acid ligands to vascular endothelial growth factor (VEGF), human neutrophil elastase, theophylline, caffeine and thrombin. The method utilized herein for identifying such nucleic acid ligands is called SELEX, an acronym for Systematic Evolution of Ligands by EXponential enrichment. Further described herein are methods for identifying highly specific nucleic acid ligands able to discriminate between closely related molecules, termed "counter-SELEX", methods for combining nucleic acids with other functional units for generation of high affinity ligands, termed "blended SELEX" and methods for preparing modified oligonucleotides capable of binding target molecules with high affinity. Specifically disclosed herein are high-affinity nucleic acid ligands to VEGF, elastase, caffeine and theophylline, including single-stranded RNA and DNA ligands, single-stranded RNA ligands

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able to discriminate between theophylline and caffeine and RNA ligands to thrombin containing 2'-NH₂-modifications.

The modified oligonucleotides of the present invention contain one or more modified nucleotide bases, which include 5-X and/or 2'-Y substitutions in pyrimidine bases and 8-X and/or 2'Y substitutions in purine bases.

Claim

1. A method for identifying nucleic acid ligands to a target, comprising:
 - a) contacting a candidate mixture of modified nucleic acids containing nucleotides chemically modified at the ribose and/or phosphate and/or base positions, but excluding modifications at the cytosine exocyclic amino, 5-bromo substitution of uracil and methylation, with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;
 - b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
 - c) amplifying the increased affinity nucleic acids *in vitro* to yield a ligand-enriched mixture of nucleic acids.
8. A purified and isolated non-naturally occurring nucleic acid ligand to vascular endothelial growth factor (VEGF), elastase, thrombin, caffeine or theophylline.
18. A method of identifying nucleic acid ligand(s) to a target molecule, comprising:
 - a) contacting a candidate mixture of nucleic acids with the target molecule, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the mixture;
 - b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture;
 - c) contacting the increased affinity nucleic acids with one or more non-target molecules, and removing nucleic acids with affinity to the non-target molecule(s); and
 - d) amplifying the nucleic acids with specific affinity to the target molecule, to yield a nucleic acid product enriched for nucleic acid sequences with higher affinity and specificity for binding to the target molecule relative to the corresponding method in which step (c) is omitted.